RESISTANCE TO *Pseudomonas solanacearum* IN *Lycopersicon esculentum*

By

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RESISTANCE TO *Pseudomonas solanacearum* IN *Lycopersicon esculentum*

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Study of a source of resistance in tomato (*Lycopersicon esculentum* Mill.) to *Pseudomonas solanacearum* E. F. Smith, the causal agent of bacterial wilt, was undertaken to evaluate the usefulness of the resistance in control of the plant disease. Plants with a high degree of resistance to *P. solanacearum* were selected from Plant Introduction 126408. These plants, however, never produced progenies with 100% survival in inoculation tests, and selections after a second generation of selfed plants were not significantly different from further selections in resistance. Resistance was expressed in progenies subjected to three different inoculation techniques in the greenhouse and was also expressed to natural populations of the pathogen in field tests in Florida, Georgia, and Panamá.

Inheritance of resistance to *P. solanacearum* of resistant selections of P.I. 126408 was characterized by crossing them to the susceptible cultivars, Bonny Best and Floradel. Segregation at the F_2 generation indicated that resistance is polygenically

inherited. Inoculation results with progenies from reciprocal crosses proved that extrachromosomal inheritance was not involved. The genes were additive with no dominance, but there was a tendency toward susceptibility in the progenies of F_2 plants. Small-fruited plants were not correlated with resistance.

At high temperatures, the resistance to F. solanacearum was still evident and was equal to that of Saturn, a cultivar with polygenic resistance. In environmentally controlled chambers, at air temperatures of 30 or 32 C, stem-inoculated plants from resistant lines did not wilt in 15 days, even though some yellowing and adventitious roots were evident. At the same temperatures, susceptible lines wilted and died within 15 days.

Tomato root exudates did not stimulate growth of F. solanacearum. Numbers of the bacterium increased in water from leachates from steamed soil or sand sterilized by filtration or in an autoclave. Living roots of resistant or susceptible plants growing in soil or sand did not increase the growth rate of bacteria in the leachates compared to the check with no plants. F. solanacearum cells did not multiply near tomato roots cultured in a mineral-water-agar medium. However, the bacterium did increase near some fungal contaminants. Such microorganisms in the soil may produce growth factors that stimulate multiplication of the bacterium.

Tomato seedlings of susceptible varieties growing asepti-

cally in a mineral-water-agar media containing P. solanacearum developed wilt symptoms. Penetration of the bacteria into the roots may have occurred at the tip of the roots as well as where secondary roots emerged. Bacteria penetrated roots of both resistant and susceptible plants.

Resistance in P.I. 126408 is protoplasmic. No differences could be detected in the development of the pathogen around resistant and susceptible roots, and invasion seemed to occur with equal frequency in each kind of plant. After entry, differences in colonization of the plant types were noted. Some development of the pathogen occurred in resistant plants after invasion, but the pathogen often did not successfully colonized the vascular tissues. Colonization of susceptible plants, however, continued until the plants were killed.

The polygenic protoplasmic resistance of P.I. 126408 may be useful in breeding programs designed to develop tomatoes resistant to bacterial wilt.

INTRODUCTION

Bacterial wilt of tomato, caused by Pseudomonas solanacearum E. F. Smith, is one of the most economically important plant diseases incited by bacteria (15). A comprehensive review of the literature on the disease before 1953 was made by Kelman (15); Buddenhagen and Kelman (4) later extended the review up to 1964. The pathogen has a wide host range and the disease occurs in almost every warm temperature, semitropical, and tropical zone of the world (15). Host plants to P. solanacearum include economic plants of the Solanaceae, such as tobacco, potato, pepper, eggplant, and tomato. Non-solanaceous plants, such as peanut and banana, are also economically damaged by strains of this organism. A total of 33 plant families contain hosts of P. solanacearum (15).

On tomato, Lycopersicon esculentum Mill., the disease has been reported predominantly from the Southeastern states of the United States. Outside the United states, losses ranging from insignificant to severe have been reported in Mexico, all countries in Central and South America, France, Holland, Italy, Switzerland, Algeria, Mozambique, Union of South Africa, Ceylon, China, Formosa, India, The Philippines, Java, Sumatra and Hawaii (15).

Detection of the organism in the field can be made by visual observation of the symptoms of the disease on host plants. Serological methods (13), and the use of a selective medium (14),

has also been employed to determine the presence of the bacteria in the field. The pathogen is soil-borne and can be found in recently cleared land as well as in cultivated land (15). Survival of the pathogen in the soil varies from place to place and from a few months to many years (15). Also, since F. solanacearum invades a wide range of plants, many of which remain symptomless (2, 3), the organism can survive on many weed hosts.

Bacterial wilt of tomato is most serious under relative high soil temperature and moisture. Vaughan (27), found that the rate of disease development increased with increased soil temperature up to 110 F. However, disease symptoms do not develop at soil temperatures below 70 F. Wilt symptoms developed more rapidly in stem-inoculated plants growing in wet rather than in relatively dry soil (27). This observation was confirmed by Gallegly and Walker (8).

The subject of root invasion by the pathogen is a controversial one. In general, wounds are thought necessary for penetration of the bacterium into the roots. These wounds may be made during transplanting, cultivation, or nematode and insect feeding on roots (15). But Van der Meer, as reviewed by Kelman (15), found that uninjured roots can be invaded by the bacterium, at least under high moisture levels. Kelman and Sequeira (17), suggested that nonwounded roots may be invaded at the points of emergence of secondary roots.

The fact that the bacterial wilt organism is soil-borne, and that it survives for many years in the soil in the absence

of crop host plants (15), makes control by crop rotation impractical. Once the organism is established in a field chemical sprays, or drenches, are of little value as a means of control of the disease. Disease-resistant crop plants seem to be the best hope for control

There are various sources of resistance to bacterial wilt in tomato, one of which seems to be determined by a single gene or a few genes. Incorporation of these resistance genes into commercially acceptable cultivars has been difficult. The association of resistance to bacterial wilt with small size of fruits (1) and the difficulties associated with transferring polygenically inherited characteristics are the major problems encountered in combining genetic resistance to P. solanacearum with production of fruits at the commercial level.

A source of resistance selected in North Carolina was found to be polygenically inherited (1). Venus and Saturn are two cultivars that have the North Carolina source of resistance to P. solanacearum (12). Even though resistance of these cultivars holds up under most field conditions (20), up to more than 90% of flowers drop under the hot field conditions of Panamá (G. Ocaña, personal communication). This observations indicates that the use of these varieties will not solve the disease problem of bacterial wilt of tomato in the tropics Ocaña (21), working with crosses made with resistant breeding material from North Carolina, found that the smaller-fruited selections were more resistant than the larger-fruited ones. Resistant selections did not yield well.

A second source of resistance was selected in Hawaii (1). Plant introduction (P.I. 127605 (L. pimpinellifolium (Jusl.) Mill.) was highly resistant under most field condition, but resistance decreased during the warm summer weather. Fruit size of some of the lines developed in Hawaii is acceptable for commercial purposes. A more recent report from Hawaii in their resistant lines indicates that "some serious breeding problems not found in the other programs were encountered with bacterial wilt" (9). Even though the reasons are not explained, the report indicates that "resistance is more effective when field temperatures are below 85 F" This may explain the poor performance of these lines reported by some investigators.

Ferrer (5), found the Hawaii lines susceptible to bacterial wilt under greenhouse conditions in Florida. Ocaña (21) and McCarter (20) also found the Hawaii lines to be susceptible under field conditions.

A selection of L. pimpinellifolium from the French Antilles, CRA-66, is also resistant under field conditions in Panamá. The line produces very small fruit and was found by Ferrer (5) to be susceptible under laboratory conditions. The source of resistance of these lines was not reported.

A line, P.I. 126408 (L. esculentum Mill.), was found by Ferrer (5) to have a higher level of resistance, in the seedling stage, than all other lines tested However, fruit which average less than 1 oz. are produced on plants of P.I. 126408, but fruit were set under relatively high temperature conditions.

Bacterial wilt resistance in tomato is highly desirable

for production of tomatoes in areas where soils are infested with the agent of the disease. Research was planned to further characterize the resistance in plants of P.I. 126408. The mode of inheritance of the resistance and some characteristics of the nature of the resistance are reported herein.

MATERIALS AND METHODS

A strain of Pseudomonas solanacearum, designated A-21, was isolated from a diseased tomato plant in Florida in 1971, and was used in all inoculation tests. It corresponds to physiological race 1 of Hayward (11). It is a highly aggressive isolate in tomato, causes wilt of tobacco when introduced in the stem or roots, and when introduced in tobacco leaves at concentrations greater than 1×10^6 cells/ml it incites a hypersensitive reaction in tobacco leaves.

Preparation of Inocula

Inoculum of P. solanacearum was prepared by streaking isolates, maintained in tubes with sterile distilled water at approx 27 ± C in the dark, onto a tretazolium medium (16). After 48-60 hr of incubation at 32 C, and based on colony appearance on this medium, isolates were transferred to nutrient broth and shaken for 20-24 hr at approx. 27 ± 2 C. Cells were centrifuged from the broth and resuspended in sterile tap water, which proved in laboratory experiences to maintain the number of living cells longer than a physiological buffered saline solution (25). The concentration of the inoculum was standardized photometrically using a "Spectronic 20" (Bausch & Lomb), spectrophotometer at a wavelenght of 600 nm to either 50, 70, or 84% transmittance. Other concentrations of inocule were derived by dilution from such standardization. A dilution

plate technique (25) was used to determine actual concentrations of bacteria in the suspensions. Fifty percent transmittance was equivalent to 2 x 10^8 cells/ml; 70%, 1 x 10^8 cells/ml; and 84%, 5 x 10^7 cells/ml.

Inoculation Techniques

Root-injury Technique

Tomato seeds were sown in 48-inch rows and 4 inches apart in soil contained in raised benches in a greenhouse. The susceptible cultivar Bonny Best was used as control. Venus, Saturn, Floradel, and selections of the P.I. 126408 were used as treatments. Rows were thinned to 50 plants/row or left unthinned according to the purpose of the test. Prior to inoculation, a furrow was made with a knife about 1 inch from the plant stems and about 2 inches deep so that one side only per line of plants was inoculated. Then 50 ml of standardized inoculum were poured along this furrow which was then closed to avoid desiccation. Three to four-week-old seedlings were inoculated using this technique.

Transplant Technique

Tomato seeds were sown in 6-inch clay pot saucers. Seven days after emergence of the seedlings, 25 ml of standardized inoculum were added to each plate. Seedlings were transplanted 5-7 days later to soil in the benches and placed in rows to keep the different lines separated. Living plants were counted

15 days after inoculation.

Stem Inoculation Technique

Tomato seedlings in the cotyledonary stage were transplanted into 4-inch pots containing steam sterilized soil. About 3 weeks after transplanting, when they were about 6 inches tall, seedlings were inoculated by piercing the hypocotyl about 0.5 cm above the soil level. The inoculum contained about 2×10^7 cells/ml. Plants were then placed in Sherer CEL 4-4, environmental chambers, at various temperatures, which were kept within 2 C of the desired temperatures throughout the experiment. A 16-hr light period/day from fluorescent and incandescent lamps giving approx. 680 lux 12 inches from the lights was used in this test. Living plants were counted 15 days after inoculation.

Tomato Plant Culture

All plants raised to maturity were planted in soil in raised benches. All secondary buds were kept pruned and the plants were trellised on strings attached to wires overhead. The plants were watered twice daily, fertilized with a 6-6-6 commercial fertilizer every 2-3 weeks, and sprayed with pesticides as needed. Steamed soil was always used in the greenhouse benches.

Crosses were made by transferring pollen to emasculated flowers. Selfing was allowed to occur naturally, since very

few flower-visiting insects or wind existed in the greenhouse.

All inoculation tests to determine levels of resistance were made in a greenhouse. Temperatures fluctuated greatly during most tests and from one test to another. During some tests, soil temperatures fluctuated between 20 and 30 C. In others, the temperatures fluctuated between 28 and 32 C.

Root Diffusates

Leachate Medium

Three tests were undertaken to assess the role of root diffusates on the growth of F. solanacearum. In the first and second tests, steamed soil was placed in 115 ml "Nalgene" disposable filter units with an 0.20μ membrane filter under the soil. Tomato plants in the first true-leaf stage were transplanted to the soil and the units were kept in a Sherer CEL 4-4 growth chamber at 28-30 C and with 12 hr of light daily. During the two weeks of plant growth, water percolated through the soil and through the sterilizing membrane. This water was collected and used as a medium for F. solanacearum.

In a third test, washed white sand was placed in steam-sterilized 4-inch pots. Tomato seeds were planted in the sand, which was watered daily with a 4-salt mineral solution with minor elements (18). Three weeks after germination of the seedlings, the sand was flooded with water and left 20 min for excess water to drain. The pots were then placed on a "Nalgene" filter unit with an 0.20μ membrane filter. Ten ml of water

were added to each pot and a vacuum of 25 inches of mercury was applied to the filter unit. The excess water from the pots was drawn through the filter and was used as a medium for F. solanacearum.

All media obtained from the pots were again filter-sterilized by forcing them through a "Swinny" filter containing an 0.20μ membrane filter. In the first two tests, half of each batch of medium was also autoclaved.

Cells of F. solanacearum, isolate A-21, were added to the soil-water medium. A 20-hr broth culture of the bacterium was centrifuged at 2,000 g to remove the bacteria. The pellet was resuspended in sterile tap water and standardized to 50% transmittance in a "Spectronic 20". Using this standard suspension, equivalent to 2×10^8 cells/ml, a suspension was obtained by dilution, containing about 1×10^3 cells in a 0.05-ml drop. The latter amount was added to each ml of soil-water medium. The tubes of media were placed on a reciprocal shaker at 30 C.

The growth of bacteria in the medium was determined by periodically diluting the medium 10-fold and planting measured volumes of the diluted medium on nutrient agar plates (25). Colonies were counted and numbers were converted to bacterial cells concentration in the original medium. Two or three dilutions were used to obtain average readings.

Mineral-Water-Agar Medium

A mineral-water-agar medium (MWA) was used to culture

tomato plants in the presence of a dilute suspension of P. solanacearum. This medium was prepared by adding Bacto-Agar (Difco) at 1.3% w/v to a modified 4-salt nutrient solution with minor elements (18). Cells of P. solanacearum, isolate A-21, from a 20-hr culture were added before solidification of the medium at 47 C. Twenty ml of this medium were placed in sterile 50-ml tubes with a cotton stopper. Tomato seeds were surface-sterilized by dipping them in 95% alcohol and then transferring them to a 10% "Clorox" solution for 2 minutes. A sterile seed was placed in each tube with the medium and the tubes were placed in a Sherer CEL 4-4 growth chamber, at a temperature of 30 ± 2 C. Plants were illuminated for 12 hr daily with fluorescent and incandescent lamps giving approx. 680 lux 12 inches from the lamps. Plants were placed about 12 inches from the lights to obtain a fair growth of the seedlings. Seedlings were observed periodically and tubes containing no seeds were used to compare growth of bacterial colonies where tomato plants were absent.

RESULTS

Tests of Inheritance

Inheritance of the resistance to bacterial wilt of plants of P.I. 126408 was investigated by screening progeny of resistant selections from this line crossed with plants of the susceptible cultivars, Monny Best and Floradel. Crosses were also made with plants of the cultivars, Venus and Saturn, which have polygenic resistance to bacterial wilt, to observe if such a cross would increase the resistance among the progeny over progeny of either parent.

Selection within P.I. 126408

Preliminary experiments demonstrated the resistance of plants of P.I 126408 to bacterial wilt, but since P.I. accessions are usually heterozygous, seed were obtained from resistant plants through successive generations in attempts to increase homozygosity. Progeny of these selections were screened for resistance.

Preliminary screening tests indicated that seedlings from the sixth selection from P.I. 126408 were the most resistant of 10 selections made and this selection was labeled 126408-6. Eight plants from 126408-6 were selected after screening progeny for resistance. Selfed seed were obtained from the 8 plants and the resulting seedlings were not significantly different from the progeny of parent selections in survival after inocu-

lation if data from all selections were lumped together. However, the progeny of selections 126408-6-2, -6-4, -6-7, and -6-8 (Table 1) were more resistant than the parent selection, and this criterion was used for further selection within those lines.

In the third generation from P.I. 126408 selections from resistant progeny of 126408-6-2 and 126408-6-8 were made after inoculations of the lines. The progeny of these selections were screened for resistance and compared with populations representing each previous generation (Table 2). Three inoculum levels of the pathogen were used.

As the inoculum level increased survival of plants decreased. At the lower level of inoculum, not all susceptible control plants, Bonny Best, were diseased. Even with the low level of inoculum resistance of selections was evident, however, by a higher percentage of living plants 2 weeks after inoculation. The data from the three levels of inoculum were lumped to evaluate the resistance of the selections.

After an analysis of variance and Duncan's multiple range test were performed on the data (Appendix 1,2), wide differences in survival of selections in inoculation tests were noted among the representatives of the generations. Resistance was increased by selection through the three generations. However, no significant differences occurred between the second and third generation. This indicated that selection in future generations would probably not significantly increase resistance in the lines.

Table 1. Progeny test of 126408-6 and selections from it for bacterial wilt resistance

Selection	Total No. Plants	Living[a)]	% Living
126408-6	24	8	33.3
126408-6-1	24	9	37.5
126408-6-2	24	16	75.0
126408-6-3	24	5	20.8
126408-6-4	24	16	75.0
126408-6-5	24	13	54.2
126408-6-6	24	9	37.5
126408-6-7	24	16	75.0
126408-6-8	24	17	79.2

a) living plants 2 weeks after root inoculation with 2×10^8 cells/ml of Pseudomonas solanacearum.

Table 2. Progeny test of selections from 126408-6 for bacterial wilt resistance

126408 Selection	50%[a] No.	50%[a] living[b]	70%[a] No.	70%[a] living[b]	84%[a] No.	84%[a] living[b]	Totals No.	Totals living[b]	%
1st gen.									
6	9	0	16	4	7	4	32	8	25.0
2nd gen.									
6-2	18	8	26	13	32	23	76	44	57.9
6-8	22	4	33	26	32	28	87	58	66.7
6-9	31	14	33	23	17	15	81	52	64.2
6-10	16	6	20	9	21	20	57	35	61.4
3rd gen.									
6-2-1	22	10	24	10	36	21	82	41	50.0
6-2-2	8	0	12	7	15	12	35	19	54.3
6-2-3	17	7	26	22	38	32	81	61	75.3
6-2-4	25	7	28	25	37	34	90	66	73.3
6-8-1	25	11	25	25	29	18	79	54	68.3
6-8-2	35	23	32	22	32	13	99	58	57.6
6-8-3	31	9	29	22	36	32	96	63	65.6
6-8-4	14	6	26	12	25	22	65	40	61.6
Bonny Best (Ck)	27	7	18	9	24	14	69	30	43.4

a) % transmittance in Spectronic 20 of bacterial suspensions used in inoculum. 50, 70, and 84% transmittance refere to 2×10^8, 1×10^8, and 5×10^7 cells/ml.

b) living plants 2 weeks after root inoculation.

Crosses with susceptible plants

The cultivar, Bonny Best, was used as a susceptible parent and crossed with a plant from 126408-6-4. A few F_1 plants were raised to maturity and these were used to obtain an F_2 population and to backcross to the resistant and susceptible parents. The data after root inoculation of the populations at two different levels of inoculum are in Table 3. Again, survival increased as inoculum level decreased. Progeny of 126408-6-2-3 had a high percentage of survival compared to the plants of the susceptible cultivar, Bonny Best. The F_1 population survival figures were intermediate between those of both parents. The F_2 population survival numbers were also intermediate between those of the parents. A backcross to the susceptible parent resulted in a population with a survival percentage below of that of the F_1.

With Floredel as the susceptible parent and 126408-6-8 as resistant parent populations were also obtained of F_1, F_2 and backcrosses to susceptible and resistant parents. Results similar to the ones with Bonny Best were obtained except the backcross to a resistant parent resulted in a higher percentage survival than that of the F_1 population, as expected (Table 4). The latter did not occur when Bonny Best was used as the susceptible parent.

Reciprocal crosses were made between Floredel and 126408-6-8 to establish if cytoplasmic inheritance was involved in the resistance. All populations from reciprocal crosses behave alike,

Table 3. Data on survival of plants from crosses of selections from F 1 126408 with Bonny Best after inoculation of two levels of inoculum of Pseudomonas solanacearum

Generation	50% b) No. plants	50% b) living[a]	50% b) % living	70% c) No. plants	70% c) living[a]	70% c) % living
Bonny Best (BB)	80	2	2.5	92	13	14.1
6-2-3* x self	63	20	31.7	67	38	56.7
F_1 (BB x 6-4*)	94	18	19.1	74	22	29.7
F_2 (BB x 6-4*) x self	83	9	10.8	77	21	27.3
Bc (6-4* x BB) 6-2-3	59	9	15.2	40	8	20.0
Bc (BB x 6-4*) x BB	73	6	8.2	67	10	14.9

* the prefix 126408 has been omitted.

a) living plants 2 weeks after root inoculation.

b) inoculum concentration: 2 x 10^8 cells/ml.

c) inoculum concentration: 2 x 10^7 cells/ml.

Table 4. Data on survival of plants from crosses of selections from F_1 126408 with Floradel after inoculation with Pseudomonas solanacearum

Line or cultivar	No. plants	living[a]	% living
Bonny Best (Control)	92	20	21.7
Floradel	79	14	17.7
126408-6-2-3 x self	78	56	71.8
F_1 (Floradel x 126408-6-8)	69	14	20.3
F_2 (Floradel x 126408-6-8) x self	58	8	13.8
Bc (126408-6-2 x Floradel) x 126408-6-2-3	66	19	28.8
Bc (Floradel x 126408-6-2) x Floradel	41	4	9.7

a) living plants 2 weeks after root inoculation with 2×10^8 cells/ml of Pseudomonas solanacearum.

which means that the resistance is probably not cytoplasmically inherited.

Progeny testing of F_2 population of the cross, Bonny Best x 126408-6-2

Variation due to increased homozygosity of genes contributing to resistance was investigated in a progeny test of an F_2 population of the cross, Bonny Best x 126408-6-2. Seeds were obtained from 49 self pollinated F_2 plants and approximately 100 seedlings from each were inoculated with F. solanacearum. The percentage survivel of the F_3 plants (Table 5) was used as an indication of the degree of resistance of the parent. Readings for plant survival were taken 3 and 6 weeks after inoculation. The selfed plants had a variation as expected for a normally distributed curve (Fig. 1 and 2). Data used to calculate expected and actual curves are in Appendix 3-4. Both curves obtained were similar and both agreed with a normally distributed population curve at the 1% level using the X^2 test. The characteristic of resistance, measured as survival percentage and which is normally distributed, indicates that this character is polygenic in nature with no dominant genes. The resistance genes have an additive effect.

Average weight per fruit of F_2 plants from the cross, Bony Best x 126408-6-4 were recorded (Table 5) and the survival percentage of progeny to bacterial wilt was correlated with fruit weight. The correlation coefficient between weight/fruit and survival percentage was found to be -0.2124 for the reading

Table 5. Survival of seedlings of 49 F_3 lines of tomato to *Pseudomonas solanacearum* and relationship to fruit weight

F_2 selection No.	No. fruits	Ave. oz./fruit	No plants	% F_3 plants living 3 weeks	% F_3 plants living 6 weeks
2	8	1.69	100	28.0	23.0
3	3	2.00	100	32.0	28.0
5	1	2.00	82	25.6	17.1
6	13	1.15	100	30.0	20.0
7	5	1.60	95	28.4	22.1
8	9	1.39	100	54.0	48.0
11	5	1.20	100	34.0	28.0
12	5	1.80	96	24.0	20.0
14	7	1.00	100	50.0	44.0
15	2	2.00	100	34.0	28.0
16	4	1.75	100	42.0	35.0
17	2	2.00	87	19.1	6.9
18	6	1.58	100	36.0	32.0
19	9	0.78	89	47.2	38.2
20	5	1.10	98	39.8	37.8
21	7	1.57	100	32.0	27.0
22	5	2.60	100	31.0	25.0
23	4	1.50	100	24.0	16.0
24	6	1.17	100	41.0	36.0
25	3	2.67	100	30.0	20.0
28	2	2.75	99	34.3	31.3

Table 5. Continued

F_2 selection No	No. fruits	Ave. oz./fruit	No. plants	% F_3 plants living 3 weeks	% F_3 plants living 6 weeks
29	4	2.50	100	21.0	12.0
30	4	1.88	100	55 0	47.0
31	3	2.50	100	22.0	16.0
33	9	1.22	100	42.0	36.0
34	5	1.20	98	17.3	14.3
35	4	1.88	99	18.2	13.1
37	3	1.50	100	14.0	13.0
38	5	1.90	100	24.0	22.0
40	5	1.60	100	27.0	20.0
41	8	1.13	100	47.0	44.0
43	4	2.13	100	54.0	46.0
44	5	1.30	100	31.0	23.0
45	8	1.38	100	27.0	19.0
46	4	1.63	90	13.1	11.1
47	3	2.00	100	36.0	30.0
48	4	1.63	60	36.2	21.7
49	7	1.21	100	51.0	50.0
50	3	1.50	97	27.8	18.6
51	10	1.45	100	47.0	42.0
52	5	1.20	100	14.0	10.0
53	4	1 75	98	28.6	21.4

Table 5. Continued

F_2 selection No.	No fruits	Ave. oz./fruit	No. plants	% F_3 plants living 3 weeks	% F_3 plants living 6 weeks
54	3	1.67	98	19.4	11.2
55	4	2.13	100	31.0	25.0
56	5	1.20	99	41.4	37.4
57	10	1.00	97	36.1	29.9
58	5	1.60	100	40.0	32.0
59	5	1.20	99	29.3	28.3
60	2	2.25	100	45.0	38.0
126408-6-2-3			100	63.0	56.0
Bonny Best			95	15.0	8.4

Figure 1. Number of F_2 selections from the cross, Bonny Best (S) x P.I. 126408 (R), categorized as to percent survival three weeks after inoculation with Pseudomonas solanacearum. (—o—o—) theoretical normal curve, (- -▲- -) actual observed frequency.

$\bar{y}$ = 32.9, s = 10.9, p = 0.75-0.50

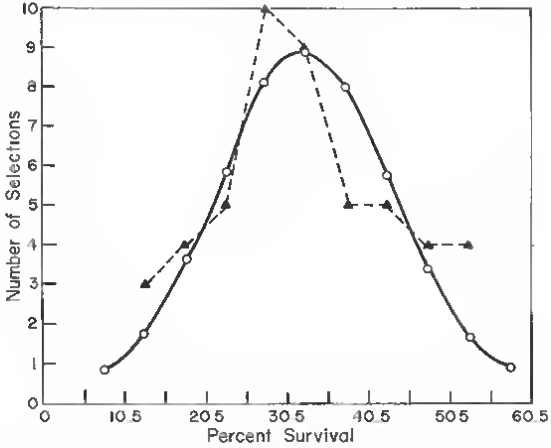
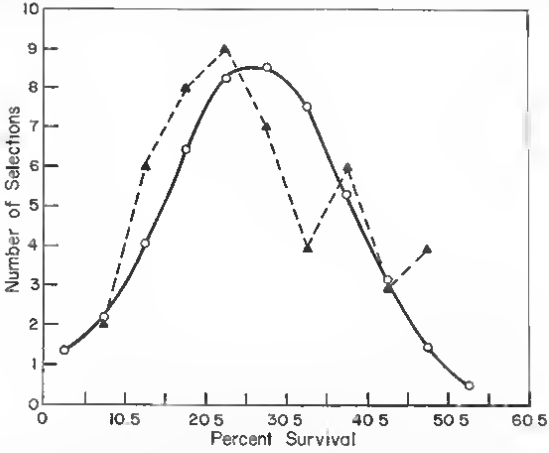

Number of Selections
0
1
2
3
4
5
6
7
8
9
10
0
10.5
20.5
30.5
40.5
50.5
60.5
Percent Survival

Figure 2. Number of F_2 selections from the cross, Bonny Best (S) x P.I. 126408 (R), categorized as to percent survival six weeks after inoculation with Pseudomonas solanacearum (-o-o-) theoretical normal curve, (- -▲- -) actual observed frequency.

$\bar{y}$ = 26.86, s = 11.5, p = 0.50-0.25


Number of Selections
Percent Survival
0
1
2
3
4
5
6
7
8
9
10
0
10.5
20.5
30.5
40.5
50.5
60.5

taken 3 weeks after inoculation. The correlation coefficient calculated 6 weeks after inoculation was -0.2566. Thus, fruit size was not correlated with resistance in the tests reported here.

Crosses between resistant cultivars and the P.I. 126408

Saturn and Venus, two resistant cultivars were used as parents in crosses with a selection from P.I. 126408 with the purpose of determining if the resistance of the two parents would be additive in the progeny. A cross between Saturn and 126408-6-4 produced an F_1 population with as many resistant plants as that of the parents (Table 6). Similar results were obtained with a cross, Venus x 126408-6-4. The F_2 population of the Saturn cross had more resistant plants than those of either parent, suggesting that genes from both parents were additive in the cross, Saturn x 126408-6-4. This increased resistance was not observed in the cross with Venus, in which the F_2 population had about as many resistant plants as the parents. A backcross to Saturn produced a population with as many resistant plants as that of the parents. These results indicate that when a selection from P.I. 126408 is crossed with Venus or Saturn, the resulting population has a resistance at least as great as that of their parents. In the cross with Saturn, resistance to bacterial wilt may have been increased through increasing homozygosity in this population.

Table 6. Data on survival of plants from crosses of selections from P.I. 126408 with Saturn after inoculation with Pseudomonas solanacearum

Line or cultivar	No. plants	living[a]	% living
Bonny Best (Control)	60	9	15.0
Saturn	60	40	66.7
126408-6 x self	60	41	68.3
F_1 (Saturn x 126408-6-4)	60	38	63.3
F_2 (Saturn x 126408-6-4) x self	60	51	85.0
Bc (Saturn x 126408-6-4) x Saturn	60	37	61.7

a) living plants 2 weeks after inoculation by the transplanting technique using 25 ml of 2 x 10^8 cells/ml of Pseudomonas solanacearum per seeded plate.

126408 Field Tests

Tests under laboratory conditions (5) indicated that certain selections of the P.I. 126408 were highly resistant to bacterial wilt caused by one isolate, A-21, of P. solanacearum. Field trials were necessary to confirm this resistance under conditions where natural populations of the pathogen exist. Three investigators cooperated in planting selections of P.I. 126408 in field trials.

S. M. McCarter, University of Georgia, Athens, planted populations of progeny of the selection 126408-7 in infested plots at Midville and Athens, Georgia (20). Only 3 out of more than 300 plants had evidence of bacterial wilt at the completion of the experiment. Plants of the susceptible check, Marion, had 81.7% mortality and plants of the selection 126408-7 had an 0.0% mortality in the test at Midville, whereas 67.9% and 2.3% mortality, respectively occurred at Athens.

G. Ocaña, University of Panamá, Panamá City, Panamá, planted 45 seedlings of a selection of the P.I. 126408-6 in a field naturally infested with P. solanacearum. All the resistant plants survived whereas all plants of the variety Floradel died.

R. Sonoda, IFAS, Agriculture Research Center, Ft. Pierce, Florida, planted individuals from 126408-6, from crosses between selections of 126408-6, and the resistant cultivars, Venus and Saturn, and individuals of the susceptible cultivars, Bonny Best and Floradel. The plots were heavily infested with P. solanacearum

since previous tomato trials in the same plots were devastated by the disease. This test was in late Spring 1973 and temperatures were above that normally occurring during tomato production. Thus, conditions were extremely favorable for disease development.

The crosses between the 126408-6-4 and the resistant cultivars had survival percentages equal to those of the resistant cultivars and a little higher than those of the selfed 126408-6 selection (Table 7). Crosses between the 126408-6 and the susceptible cultivars Bonny Best and Floradel produced F_1 populations just as susceptible as the susceptible parents.

Results of the Ft. Pierce test were consistent with the seedling inoculation tests. Crosses of 126408 with the resistant Venus and Saturn do not decrease resistance in the progeny, but crosses with the susceptible Bonny Best and Floradel do decrease resistance in progeny.

Nature of Resistance

Studies of the nature of the resistance in plants from P.I. 126408 were undertaken to gather information on the nature of the resistance of these plants for future use of this resistance in field control of bacterial wilt.

Root Diffusates

Experiments were undertaken on the role of root diffusates on multiplication of *P. solenacearum* around the roots of plants resistant and susceptible to bacterial wilt. Root diffusates

Table 7- Field evaluation of resistance to Pseudomonas solanacearum of crosses between susceptible and resistant cultivars with selections from P.I. 126408 at Ft. Pierce, Fla.

Cultivar, line or cross	No. plants transplanted	No. plants living[a]	% living
Bonny Best	56	11	19.6
Venus	56	34	60.7
Saturn	56	32	57.1
Floradel	56	18	32.1
126408-6 x Bonny Best	56	6	10.7
Venus x 126408-6-4	56	32	57.1
Saturn x 126408-6-4	56	31	55.6
Floradel x 126408-6-2	56	12	21.4
126408-6 x self	56	25	44.6

a) living plants 4 weeks after transplanting in naturally infested soil

have been found to be important in development of some other diseases involving root pathogens. Root exudates play an important role in the germination of spores or solerotia of many fungi, chemotaxic responses such as in the case of Phytophthora spp., and maintaining growth of pathogens in the rhizosphere (23).

Leachate test

Root diffusates were collected in water from pots containing plants resistant to bacterial wilt, Saturn and 126408-6-2-3, and plants susceptible to bacterial wilt, Bony Beet. Controls consisted of steamed soil or white sand without plants.

In three tests, P. solanacearum grew in the control medium, except in one treatment (Table 8). Since only mineral nutrients were added to the medium and no source of carbon was added, it was felt that the growth factors released in the soil probable were of microorganism origin. Since P. solanacearum grew in leachates from washed white sand, probably algae or other autotrophic organisms were involved in the stimulation of growth of the bacteria.

The growth of P. solanacearum in the leachate media was relatively slow, compared to growth of the bacteria in nutrient broth. Populations of this organism reach 10^9 cells/ml in 48 hr in nutrient broth (5). Therefore, if growth factors were released from the tomato roots, growth of the bacteria in the media from pots with plants should have increased over the

Table 8. Influence of substrate leachates on growth of Pseudomonas solanacearum in vitro

Water extracted from substrate + or - plant		Bacteria/ml of substrate leachates Hr 0	24	48
Test 1				
Bonny Best	F (a)	5.0×10^2	3.0×10^6	2.9×10^7
	FA (b)	1.0×10^3	2.7×10^6	1.8×10^7
126408-6-8	F	2.4×10^2	2.5×10^5	1.7×10^7
	FA	2.9×10^2	3.1×10^5	1.9×10^7
Soil only (Ck)	F	5.0×10^2	0	0
	FA	2.2×10^2	2.1×10^5	2.6×10^6
Test 2				
Bonny Best	F	1.8×10^3	4.8×10^6	3.0×10^7
	FA	1.5×10^3	8.2×10^6	3.7×10^7
126408-6-8	F	1.6×10^3	5.2×10^6	1.1×10^7
	FA	1.6×10^3	8.7×10^6	1.5×10^7
Soil only (Ck)	F	1.1×10^3	6.0×10^5	6.5×10^6
	FA	1.3×10^3	3.1×10^5	5.7×10^6
Test 3				
Saturn	F	1.1×10^3	2.8×10^6	1.8×10^7
Bonny Best	F	9.0×10^2	4.0×10^6	1.8×10^7
126408-6-2-3	F	1.1×10^3	2.7×10^6	1.8×10^7
Sand only (Ck)	F	1.2×10^3	3.4×10^6	1.4×10^7

(a) filter-sterilized

(b) filter-sterilized and autoclaved

control pots. In no instance, however, did increased growth occur in media from pots containing tomato plants (Table 8). Therefore, it can be concluded that diffusates from the tomato plants had no effect on the observed growth of F. solanacearum in the aqueous medium.

Mineral-water-agar test

The above experiments had a limitation of possible dilution of released growth factors from roots to the point of making detection impossible. A different approach was used in an attempt to determine whether factors that might influence growth of F. solanacearum are released in minute amounts from roots. Bacterial cells were added to a mineral-water-agar (MWA) medium at a concentration of approximately 1×10^3 cells/ml. After 4-5 days, minute colonies, 0.1 mm in diam, were visible with a binocular microscope at 100 X and also with the unaided eye when the tube containing the medium was placed against a source of light, such as a fluorescent lamp. The colonies did not increase in size over the next two months, when the temperature remained at approximately 30 C.

Seeds of two cultivars, Saturn (resistant), and Bonny Best (susceptible), and the line 126408-6-2-3-Bk were placed on the agar surface after colonies of F. solanacearum had become visible. Germination of seed and growth of the plants occurred on this medium. Colonies of F. solanacearum, near or far from the roots never enlarged beyond the size of colonies in tubes without plants. However, in about 5% of the tubes, fungal

contaminants occurred, and the size of F. solanacearum colonies near these fungal colonies did increase in size. The contaminant fungi were not identified. This was considered as further evidence that tomato root diffusates do not affect the growth of F. solanacearum, but possibly microorganisms in the soil do produce growth factors that effect multiplication of the bacterium.

Penetration of Noninjured Roots

Preliminary experiments in which inoculum of F. solanacearum was poured over roots of tomato plants growing in vermiculite indicated that the pathogen could penetrate noninjured roots (Table 9). This observation was confirmed in the NWA medium.

Penetration of noninjured roots of resistant and susceptible tomato seedlings occurred when 100 or 1,000 cells/ml of F. solanacearum were added to the medium (Table 10). At the higher density of bacterial colonies, both resistant and susceptible plants had browned roots. Browning symptoms were observed at the root tips and areas where secondary roots emerged in both resistant and susceptible plants. Constriction of stems and wilting of seedlings were also observed, as well as development of adventitious roots in some cases in both types of plants.

In the absence of mechanical injuries, and assuming that openings in the roots are necessary for ingress, the infection court could theoretically be the places where secondary roots

Table 9. Bacterial wilt development in plants with noninjured roots growing in vermiculite[a)]

Cultivar or line	No. plants	living[b)]	% living
Venus	10	10	100.0
Bonny Best	12	4	33.3
126408-6-9	12	10	84.0
126408-6-2-1	12	11	91.7
126408-6-8-1	12	11	91.7

a) roots were drenched with suspensions of 2 x 10^8 cells/ml of P. solanacearum.

b) living plants after 2 weeks after addition of bacterial suspension to the substrate.

Table 10. Penetration of tomato roots by Pseudomonas solanacearum in a mineral-water-agar media

Line or cultivar	total plants	% living[a)]	% with brown roots
Test 1 *			
Bonny Best	16	37.5	100.0
Test 2 **			
126408-6-2-3-Bk	7	57.1	100.0
Bonny Best	6	33.3	100.0

a) living plants 6 weeks after germination in MWA.

* inoculum concentration = 100 cells/ml.

** inoculum concentration = 1,000 cells/ml

emerge. Movement of bacteria in this environment is restricted, therefore, they had to be present at the site of injury for penetration. The probability of a bacterium being exactly at the site where a secondary root emerged was calculated. At the density of 1,000 bacteria per ml in the medium, 3 out of 10^9 times a bacterium would be in the area where a secondary root emerged (Appendix 5) Observed results utilizing the MWA technique did not fit the probability, since invasion occurred far more frequently than the probability dictated.

To account for such a high rate of invasion of plants without artificial injures, it can only be speculated that the pathogen might have multiplied on the surface of the root and that populations of the pathogen near the secondary root emergence sites might have been much greater than could be determined by the techniques used in these experiments.

Evidence was found that the bacterium might multiply extensively near the root tip. In several instances, turbidity was noticed around root tips, in the case of the susceptible cultivar Bonny Best, which then became brown and ceased to grow. In isolations made after carefully removing root tips from the agar medium, only colonies of P. solanacearum could be recovered. That the bacterium multiplies on root tips and enters them directly can only be suggested at this time. More refined techniques must be developed before multiplication and entry of P. solanacearum into root tips can be established with certainty.

Effect of Temperature on Resistance

Resistance to bacterial wilt of some tomato lines is affected by temperature (9). For this reason plants of 126408-6-2-3-Bk were tested for resistance at various temperatures. Plants of Saturn and Bonny Best were included, respectively, as resistant and susceptible controls. Plants were inoculated by the stem-inoculation technique and placed at 25, 28, 30, and 32 C after inoculation. Almost all of the three types of plants developed some symptoms of the disease, such as adventitious root formation on the stem and some degree of dwarfing or vascular discoloration, but very little wilting occurred in any of them at 25 and 28 C, even among plants of the susceptible Bonny Best (Table 11). Plants of Saturn and 126408-6-2-3-Bk remained resistant at 30 and 32 C whereas those of Bonny Best were almost totally susceptible. Since Saturn remains resistant to bacterial wilt even under some field conditions where all susceptible plants die, plants of 126408-6-2-3-Bk will probably hold their resistance under the same field conditions.

This experiment also demonstrated that resistance of the P.I. 126408 plants operates even after the pathogen had been introduced into the stems.

Table 11. Influence of temperature on bacterial wilt development on resistant and susceptible tomato plants

Line or cultivar	Temperature C			
	25	26	30	32
Bonny Best	5/8 *	6/8	1/8	0/8
126408-6-2-3-Bk	7/8	7/8	8/8	8/8
Saturn	8/8	8/8	8/8	7/8

* healthy plants/total inoculated using the stem injury technique with 2 x 10^7 cells/ml of Pseudomonas solanacearum 15 days after inoculation.

DISCUSSION

Inheritance of the resistance to bacterial wilt of plants of the tomato line P.I. 126408 was observed in crosses with susceptible cultivars. Under greenhouse conditions, where the observations were made, soil temperature and inoculum levels greatly influenced the survival of tested lines. But in a given experiment, where different lines were exposed to the same environmental conditions, the higher survival rate occurred in lines with the higher level of resistance. From the cross, Bonny Best (susceptible) x 126408 (resistant), the survival percentage of the F_1 progeny was between that of the parents. Probably no dominance occurred but there was a slight tendency toward the susceptible side in the population observed. The levels of resistance in the F_2 population followed a curve close to a normal curve. The significance of these data is that it confirms that the resistance behaves as expected for a polygenically inherited character with an additive gene effect.

The possibility of extrachromosomal inheritance was discarded by making reciprocal crosses with the susceptible cultivar Floradel and the P.I. 126408. The results were that when either parent was used as female, the F_1 progeny behaved the same. In both cases, the resulting survival percentage was between that of parents, but again a little toward the susceptible parent.

The back crosses to both parents did not always behave as expected for a polygenically inherited character, i.e., the survival percentage did not fall between that of the F_1 population and their respective recurrent parent. A possible explanation is that the resistant parents were not selfed with the specific purpose of achieving complete homozygosity. It was assumed that they were nearly homozygous for the character of resistance from the results of inoculation tests. Variation among resistant lines used as parents were detected, but this variation was difficult to measure accurately and it was disregarded as not significant.

Robinson (22) found that measuring resistance of potato varieties to F. solanacearum was a difficult task. Some potato lines were resistant and later became susceptible and these were considered to have vertical resistance (22). According to Robinson, a high mutability of the pathogen accounted for the poor performance of potato lines thought to have had vertical resistance. Because of this Robinson suggested that horizontal resistance, in this case of resistance to F. solanacearum, has much greater agricultural value.

In all crops in which resistance to F. solanacearum, has been found, stable resistance has been determined to be inherited in a polygenic manner (15). Transfer of this resistance to economically accepted cultivars has been slow. The reason for this is because in each cross to susceptible plants with commercial quality, approximately half of the genes governing resistance are replaced in the F_1 generation.

Some of them can be recovered by careful selection for resistance in the F_2 population. In practice, however certain levels of resistance are usually lost with each cross.

Polygenic resistance is affected by environmental factors to a greater degree than vertical resistance (26). In the work reported here, resistance was still present at 32 C, but one would expect both temperature and moisture levels to greatly effect the degree of resistance. Also, such factors as solar radiation (22), fertility (7), and certain nematode damage (19) may alter the level of resistance.

French (6), and Gonzales et al (10), working on potato with a polygenic resistance, found that selection of clones from a cross between resistant and susceptible plants can be done best by planting inoculated potato plants under lower temperature conditions in the field where resistance is best expressed. Work reported here suggests that selections can best be made of tomato segregating lines where polygenic resistance is involved at about 30 or 32 C.

The level of resistance of plants of P.I. 126408 to bacterial wilt does not appear superior to the North Carolina resistance that occurs in the cultivars Saturn and Venus. However, the resistance appears equally as effective. Therefore, one wonders about the usefulness of the resistance in future commercial application.

The resistance might be useful in crosses with Venus and Saturn to obtain relatively large-fruited lines that will set fruits in tropical climates. P.I. 126408 was originally

collected in Panamá (24), and seems to set many fruits under relatively high temperatures. Crosses of Saturn or Venus with susceptible lines to obtain good fruit-setting properties under high temperatures results in decreased bacterial wilt resistance (21). But crosses of these cultivars with P.I. 126408 would not decrease resistance to bacterial wilt and selection for fruit setting ability could be made in the progeny without fear of losing the level of resistance to bacterial wilt .

Small-fruit size has often been associated with bacterial wilt resistance using the North Carolina resistance (1, 21). In the F_2 population from the cross, Bonny Best x 126408, no correlation of fruit size and bacterial wilt resistance was noted. This is encouraging, but not necessarily conclusive evidence that fruit-size and bacterial wilt resistance are not linked in the P.I. 126408 plants. However, if this is correct, the bacterial wilt resistance of P.I. 126408 may be incorporated into large-fruited lines with much less effort than was the case with the North Carolina resistance.

The nature of bacterial wilt resistance in the P.I. 126408 is protoplasmic. Experiments designed in this work have failed to show any differences in the effect of the resistant plants on growth of the bacterium in the medium around the roots. The bacterium also seemed to invade resistant plants as effectively as susceptible plants. Invasion occured in both types of plants without artificial injury. Resistance seems to involve mechanisms that reduce colonization of the host after invasion. After invasion, the pathogen seems to

spread slowly, and then gradually declines in colonization of the host tissues. In susceptible plants, however, the pathogen does not decline in colonization, but continues to grow within infected plants until wilting and death of the plants occure. The mechanism of resistance in plants of P.I. 126408 seems to be similar to that of the North Carolina resistance.

Breeding for resistance to bacterial wilt of tomato is possibly the best way to control the disease under field conditions, but problems of inheritance of all sources of resistance available make it clear that development of commercially acceptable cultivars will be difficult. There is still need for a source of resistance to minimize problems in breeding for disease resistance to bacterial wilt in tomato so that resistant varieties with a high degree of resistance can become available for commercial use. Finding a source of resistance to bacterial wilt in tomatoes which could be easily transferred to commercial varieties is still of foremost importance.

SUMMARY

Selections from P.I. 126408 were found that had a high level of resistance to bacterial wilt of tomato. The resistance level was increased by selection, but not significantly so after the second generation of selfing. The resistance was evident in experiments involving three inoculations techniques and was present to natural populations of the pathogen in field tests in Florida, Georgia, and Panamá.

Progeny of crosses of the susceptible cultivars Bonny Best and Floradel, with selections from P.I. 126408, established that the resistance was inherited in a polygenic manner. Reciprocal crosses with Floradel produced progenies similar in their resistance to P. solanacearum, suggesting that the resistance was not extrachromosomal. Progenies of crosses with resistant cultivars Venus and Saturn, with selections from P.I. 126408, were as resistant as either parent.

The bacterium multiplied in leachates from steamed soil and washed-white sand. Increased multiplication was not noted in leachates from soil or white sand that contained living roots of resistant or susceptible tomato plants. Diffusates from tomato roots growing in a mineral-water-agar medium containing cells of P. solanacearum did not influence growth of the bacterium. Some fungal contaminants, however, did result in increased growth of the bacterium in the medium.

The pathogen entered roots that were not artificially injured. Entry may have occurred where secondary roots emerged

but the probability calculated for such entry was much less than that actually observed.

Observations made suggest that the bacterium multiplies near the root tips and that entry occurs on living nonwounded. No differences were found between invasion of resistant or susceptible plants.

The resistant plants from P.I. 126408 were as resistant as Saturn at 30 and 32 C. Very little wilting occurred at 25 or 28 C, even in susceptible plants.

Observations suggest that resistance of the P.I. 126408 plants was protoplasmic in nature and was expressed during colonization of the host. The resistant plants are not colonized at the same rate as susceptible plants.

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APPENDICES

APPENDIX 1. Analysis of variance of data on resistance of plants in three generations from P.I. 126408

Source	DF	SS	MS	F	Prob.> F
Selection	2	0.4775	0.2387	3.456	0.0434
Residual	30	2.0725	0.0691		
Inoculum	2	2.1232	1.0616	15.367	0.0001
Residual	30	2.0725	0.0691		
Selection-Inoculum	4	0.0031	0.0508	0.735	0.5775
Residual	30	2.0725	0.0691		

APPENDIX 2. Duncan's multiple range test of significance of differences in resistance among generations for P.I. 126408

Duncan's Test

Generation of Selection	Mean Transformed	Mean Original[1)]
3	0.7042	0.6062 a
2	0.6975	0.6097 a
1	0.2870	0.2738 b

1) Figures with same letter are not significantly different.

APPENDIX 3. Observed frequency of selections compared to e) normal curve

Range 1)	Sd 2)	Z values 3)	Area 4)	exp. no. 5)	obs. 6)	χ^2
0-10.5	2.51	.4940	.0197	0.97	0	0.97
10.5-15.5	2.06	.4803	.0351	1.72	3	0.95
15.5-20.5	1.60	.4452	.0723	3.54	4	0.06
20.5-25.5	1.14	.3729	.1212	5.94	5	0.15
25.5-30.5	0.68	.2517	.1646	8.07	10	0.46
30.5-35.5	0.22	.0871	.1819	8.91	9	0.00
35.5-40.5	0.24	.0948	.1632	8.00	5	1.13
40.5-45.5	0.70	.2580	.1190	5.83	5	0.12
45.5-50.5	1.16	.3770	.0693	3.40	4	0.11
50.5-55.5	1.16	.4463	.0345	1.69	4	3.16
55.5-60.5	2.07	.4808	.0192	0.94	0	0.94

e) 3 weeks after inoculation with 2 x 10^8 cells/ml of Pseudomonas solanacearum.

1) Range corresponding to percent living.

2) Standard deviation from mean.

3) Z values corresponding to 5% range.

4) Area of normal curve.

5) Expected frequency of selections per area.

6) Observed frequency of selections per area.

APPENDIX 4. Observed frequency of selections compared to a normal curve[b)]

Area[1)]	Sd.[2)]	Z values[3)]	Area[4)]	Expected[5)]	Actual[6)]	x^2
0-5.5	1.91	.4719	.0281	1.38	0	1.38
5.6-10.5	1.46	.4279	.0440	2.16	2	.12
10.6-15.5	1.02	.3461	.0818	4.01	6	.99
15.6-20.5	0.57	.2157	.1304	6.39	8	.41
20.6-25.5	0.12	.0478	.1679	8.23	9	.07
25.6-30.5	0.32	.1255	.1733	8.49	7	.26
30.6-35.5	0.77	.2794	.1539	7.54	4	1.66
35.6-40.5	1.22	.3888	.1094	5.36	6	.76
40.6-45.5	1.67	.4525	.0631	3.12	3	.46
45.6-50.5	2.12	.4830	.0305	1.49	4	4.23
50.5-55.5	2.56	.4948	.0118	0.58	0	.58
55.6-60.5	3.01	.4987	.0039	.19	0	.19

b) 6 weeks after inoculation with 2 x 10^8 cells/ml of Pseudomonas solanacearum

1) Range corresponding to percent living.

2) Standard deviation from mean.

3) Z values corresponding to 5% range.

4) Area of normal curve.

5) Expected frequency of selections per area.

6) Observed frequency of selections per area.

APPENDIX 5. Calculations used to figure probability of a bacterium being at injury caused by emergence of secondary root.

Bacterial size = 2,0 x 0.5

Root diameter = 0.25 mm

Bacterial density in agar = 1 cell/mm^3

Formula = $\frac{1}{a} \times \frac{1}{b} \times \frac{1}{c}$ = probability

a = No. of bacterium diam in an mm^2 = 2000

b = No. of root diam in an mm^2 = 16

c = probability of a secondary root touching ane bacterium at the point of emergence

$$= \frac{\text{No. of cells in area occupied by root}}{\text{total cells in } mm^3}$$

$$\frac{1}{2000} \times \frac{1}{16} \times \frac{1}{10^4} = 3.1 \times 10^9$$

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

R E Stall

R. E. Stall, Chairman
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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D. A. Roberts
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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S. J. Locascio
Professor of Vegetable Crops

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1974

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